Journal of Neurocytology **16**, 333–345 (1987)

An electron microscopic study of GABAergic neurons and terminals in the central nucleus of the inferior colliculus of the rat

ROSALINDA C. ROBERTS* and CHARLES E. RIBAK†

Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717, USA

Received 27 May 1986; revised 20 October and 18 December 1986; accepted 23 December 1986

Summary

Neurons and terminals in the ventral lateral portion of the central nucleus of the inferior colliculus (ICCN) of the rat were labelled immunocytochemically with antisera to GABA or to its synthesizing enzyme, glutamic acid decarboxylase. Four types of GABAergic neuron are described: small, medium-sized and large multipolar neurons, as well as medium-sized bipolar neurons. All sizes of GABAergic multipolar neurons are characterized by highly infolded nuclei, many mitochondria and both asymmetric and symmetric axosomatic synapses. A dense plexus of terminals occurs on the proximal dendrites of GABAergic neurons, and most of these terminals form asymmetric axodendritic contacts. Small GABAergic neurons (diameter $< 15\,\mu m$) are multipolar, and have a large nucleus to cytoplasm ratio, prominent nucleoli and usually two to five axosomatic synapses per thin section, with the majority of these contacts being symmetric. Medium-sized GABAergic neurons (15–25 μm in diameter) display both multipolar and fusiform shaped somata, have a more abundant cytoplasm than the small neurons and show about ten axosomatic contacts per thin section. Large GABAergic neurons (diameters $> 25\,\mu m$) have eccentrically located, highly infolded nuclei, abundant cytoplasm and a denser plexus of terminals that form axosomatic synapses than the other cell types. These results indicate that four of the six major cell types in the ICCN are probably GABAergic inhibitory neurons.

The axon initial segments of GABAergic neurons in the ICCN all have similar features in that they are contacted by only one or two terminals that form symmetric synapses on their proximal portions and are invested by a glial sheath from 3 to 20 μm from the cell body. Many immunoreactive myelinated axons (approximately 0.5 μm in diameter) are observed and some terminals that arise from these axons form synapses with small neuronal somata. Both these and other labelled terminals are shown to form symmetric synapses. These data suggest a complex circuitry for the GABAergic neurons within the ICCN.

Introduction

Previous studies indicate that GABA is a major inhibitory transmitter in the inferior colliculus (IC). Biochemical studies show that the IC contains high levels of GABA and its synthesizing enzyme, glutamic acid decarboxylase (GAD) (Tachibana & Kuriyama, 1974; Fisher & Davies, 1976; Contreras & Bachelard, 1979). Iontophoretic application of GABA produces an inhibitory effect in neurons of the IC (Watanabe & Simada, 1973; Faingold *et al.*, 1985) which is enhanced by simultaneous application of the benzodiazepine, flurazepam, or the GABA-uptake inhibitor, nipecotic acid. Also, immunocytochemical studies have described the existence of numerous GAD-positive immunoreactive neurons and processes in the IC at the light microscopic level in rats and gerbils (Roberts *et al.*, 1984, 1985a, b; Vetter & Mugnaini, 1984; Mugnaini & Oertel, 1985). These studies have described a heterogeneous population of GAD-positive neurons in all subdivisions of the IC as well as GAD-positive puncta, which probably represent axon terminals and small transversely sectioned preterminal axons and dendrites. The immunoreactive neurons were identified on the basis of their dendritic orientation and the shape of their somata. Most of the GAD-positive neurons in the IC were small and medium-sized multipolar neurons. Other cell types included large multipolar neurons with somal diameters greater than 25 μm and

**Present address*: Department of Neurology, Massachusetts General Hospital, Boston, MA 02114, USA.
†To whom correspondence should be addressed.

0300-4864/87 $03.00 + .12

medium-sized fusiform neurons (somal diameters between 15 and 25 μm) with bitufted dendrites that resembled disc-shaped neurons.

Other immunocytochemical studies have localized GAD in lower brainstem auditory nuclei. Both GAD-positive cell bodies and axon terminals have been reported in the cochlear nucleus and the dorsal and ventral nuclei of the lateral lemniscus of the cat (Adams, 1984; Adams & Mugnaini, 1984, 1985a), in the dorsal cochlear nucleus of the rat (Mugnaini, 1985) and in the superior olivary complex and cochlear nuclear complex of the gerbil, rat and guinea pig (Moore & Moore, 1984; Roberts *et al.*, 1985a, b; Thompson *et al.*, 1985). Certain brainstem auditory nuclei may be the source of some of the GAD-positive terminals in the IC because most neurons in the dorsal nucleus of the lateral lemniscus are GAD-positive and probably project to the IC (Adams & Mugnaini, 1984; Mugnaini & Oertel, 1985). Similarly, neurons that resemble projection neurons in the lateral superior olive are also GAD-positive and they may also project to the IC (Moore & Moore, 1984; Roberts *et al.*, 1985a).

The purpose of the present study was to examine the electron microscopic features of the GABAergic neurons in the IC of the rat. Recently, we have described the ultrastructural features of six types of neuron in the central nucleus of the IC of the rat as a basis for the present analysis (Ribak & Roberts, 1986). The present study has concentrated on a description of GABAergic neurons and terminals in the ventral lateral portion of this brain region. This work is pertinent to our studies on the genetically epilepsy prone rat (GEPR) which displays a significant increase in the number of GABAergic neurons in the IC as compared to the non-seizing Sprague–Dawley rat (Roberts *et al.*, 1985b). Since this increase was most dramatic in the ventral–lateral portion of the central nucleus, this region was the focus of the present study to gain a better understanding of the normal neuronal circuitry of GABA neurons.

Methods

Seven adult Sprague–Dawley rats obtained from Simonsen Laboratories (Gilroy, CA) were used in this study. Three of the animals received a 10 μl injection of a 1.0% colchicine solution into the lateral ventricle 24 h prior to sacrifice (Ribak *et al.*, 1978). All animals were deeply anaesthetized with Nembutal and transcardially perfused according to one of three protocols. Two of the animals pretreated with colchicine were perfused according to procedure 1 (Zahm *et al.*, 1985). Initially, 30–50 ml of phosphate-buffered saline (PBS) containing 2·5% sucrose and 0·5% procaine were perfused to clear all vessels of blood cells. Then 300 ml of the fixative, containing 4% paraformaldehyde, 0.1% glutaraldehyde and 2.5% sucrose in 0.07 M phosphate buffer (pH 7.4, 25° C), were perfused for about 15 min followed by 100 ml of PBS and 2.5% sucrose. Following the perfusions, the inferior colliculi were dissected out and placed in PBS and 15% sucrose until they sunk. The excess sucrose was blotted off the tissue prior to its immersion in liquid nitrogen. The blocks were then brought to room temperature and sectioned on a vibratome.

Other rats pretreated with colchicine were perfused according to a second procedure (Mugnaini, 1985) which has four stages: (i) 100 ml per rat of Ringer's solution containing 0.85% NaCl, 0.25% KCl and 0.02% $NaHCO_3$ (pH 7.3; 37° C); (ii) 300 ml per rat of 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M phosphate buffer (pH 6.9; 25° C); (iii) 100 ml per rat of 0.1 M phosphate buffer (pH 7.2; 25° C); (iv) 500 ml per rat of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2; 25° C). Three other rats which did not receive colchicine were perfused according to a third protocol (Mugnaini, 1985). These animals were perfused with 100 ml per rat of Ringer's solution followed by 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2; 25° C), and then by 500 ml per rat of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2; 25° C). The animals were placed in the refrigerator and the brains were removed the next day.

The inferior colliculi from all animals were sectioned on an Oxford Vibratome in the coronal plane at a thickness of 50 μm. Blocks were cut from the ventral–lateral portion of the central nucleus of the IC from sections obtained from the middle of the rostrocaudal axis. This region consists of the entire ventral half of the ICCN shown in the middle diagram of the frontal series in Fig. 11 from Faye-Lund & Osen (1985). The tissue was then incubated in anti-GAD serum (Oertel *et al.*, 1981) or anti-GABA serum (Immunonuclear Co.) followed by subsequent incubations in reagents obtained from an avidin–biotin peroxidase kit (Vectastain, Vector Laboratories, Inc.). The details of these methods have been described previously (Roberts *et al.*, 1985b).

These tissue blocks were postfixed in 1.0% OsO_4 for 30–60 min, dehydrated in ethanol and embedded in Epon 812. Semithin (1–3 μm) sections were cut from embedded specimens and examined with a light microscope for immunostaining. Serial thin sections were cut on a Sorvall ultramicrotome, mounted on Formvar-coated slot grids, stained with uranyl acetate and lead citrate and examined with the electron microscope.

Immunoreactive somata, dendrites and axons were identified by the presence of electron-dense reaction product associated with cytoplasmic organelles. Since reaction product was invariably associated with the plasma membranes of these profiles, symmetric synapses at such sites were often difficult to identify with certainty. The criteria used to identify a synapse included: a few synaptic vesicles clustered at the presynaptic membrane, parallel pre- and postsynaptic membranes, and increased electron density in the synaptic cleft. In some cases the identification of these features was hindered by the presence of immunoreaction product.

Results

Several morphological types of GABAergic neurons, puncta and processes were identified in semithin

plastic-embedded sections, 1 μm thick, with the light microscope and in adjacent thin sections with the electron microscope. GAD and GABA immunocytochemical preparations revealed similar types of labelled neurons and terminals. However, more labelled somata were observed in the GABA preparations than in the GAD preparations. This observation is not unusual as this phenomenon has been previously noted when comparing GAD and GABA material of cerebral cortex (Somogyi *et al.*, 1984). In general, the colchicine preparations revealed more labelled somata and fewer numbers of labelled terminals than preparations from non-colchicine-treated animals.

GABAergic somata and proximal dendrites

Most of the GABAergic neurons in the ventral–lateral portion of the central nucleus of the inferior colliculus (ICCN) are less than 25 μm in diameter, have round somata and display dendrites that radiate from the cell body in several directions. These data are consistent with previous light microscopic immunocytochemical results (Roberts *et al.*, 1984, 1985a, b; Vetter & Mugnaini, 1984; Mugnaini & Oertel, 1985; Thompson *et al.*, 1985). The most frequent of these multipolar GABAergic neurons has a somal diameter of less than 15 μm. This type of neuron has infolded nuclear membranes, a prominent nucleolus and a large nucleus to cytoplasm ratio (Fig. 1b). Usually, two to five terminals contact its soma in any given section, and most of them form symmetric synapses and contain flattened or pleomorphic vesicles. Typically, immunoreactive terminals form symmetric synapses adjacent to non-immunoreactive terminals that form asymmetric synapses (Fig. 1A). The dendrites from small GABAergic cells were often followed for 30 μm from the soma and received a moderate number of axodendritic synapses, most of which were asymmetric. Spines are not present on these proximal dendritic segments.

Medium-sized (15–22 μm in diameter) GABAergic neurons are also very abundant in the ICCN. Most GABAergic neurons in this size category have round somata and dendrites that radiate from various poles of the soma (Fig. 2A). These neurons share some of the features of the small neurons in that they have infolded nuclear membranes, many mitochondria and a denser axonal plexus that contacts its proximal dendrites than that which contacts its soma. However, this type of neuron has considerably more axosomatic contacts in a single section than small neurons (Fig. 2C). For example, the round soma in Fig. 2A is contacted by eight terminals and the majority of them form symmetric synapses and contain flattened or pleomorphic synaptic vesicles (Fig. 2C). In addition, the nucleus to cytoplasm ratio is not as large as in the small neurons.

The ICCN also contains a small population of medium-sized neurons (15–25 μm in diameter) with fusiform somata and stout bipolar dendrites (5–6 μm in diameter). These neurons have infolded, centrally placed nuclei and many mitochondria (Fig. 3A, B). The pattern and number of axosomatic synapses are similar to those for the medium-sized neurons with round cell bodies. For example, the fusiform soma in Fig. 3B is contacted by 12 terminals in this thin section. Both terminals that form symmetric synapses (eight) and have flattened or pleomorphic vesicles, as well as those that form asymmetric synapses (four), contact this soma (Fig. 3B, C). It is interesting to note that some of these terminals which form axosomatic contacts tend to cluster in groups of three or four (Fig. 3C). In contrast to the soma, the proximal dendrites are contacted by a dense plexus of terminals, and many of these terminals form asymmetric synapses and have round vesicles (Fig. 3D, E).

A small population of large GABAergic neurons exists in the ICCN. These neurons have round somata and dendrites that radiate from the cell body in several directions (Fig. 4A). The nuclear membranes are highly infolded and the nucleus is eccentrically located. The perikaryal cytoplasm contains many mitochondria, a finding that is similar for the other types of GABAergic neuron. The large neurons have a dense plexus of terminals associated with their somal surface, and most of these terminals form *symmetric* synapses. Another dense plexus of terminals contacts the proximal dendrites, and it is interesting to note that most of these terminals form *asymmetric* synapses. The proximal dendrites are 5–6 μm in diameter at the point where they are in continuity with the soma. Large neurons typically display many lysosomes within their somata, but these organelles appear to accumulate in the proximal dendrites in those neurons obtained from colchicine-treated preparations (Fig. 4A). This phenomenon which was described previously for other brain regions (Gorenstein *et al.*, 1985; Gorenstein & Ribak, 1985) is caused by the colchicine treatment, and is more obvious in the large neurons because they have many more lysosomes than the smaller neurons (Ribak & Roberts, 1986).

GABAergic dendrites

Profiles of immunoreactive dendrites are observed frequently in the neuropil and they display diameters that range in size from less than 0.5 μm to approximately 6 μm. The immunoreactive dendrites are contacted predominantly by terminals that form asymmetric synapses and contain round vesicles (Figs 3D, 3E, 4B). However, many examples of terminals that form symmetric synapses and contain

immunoreactivity are also observed (Fig. 5E). In contrast to the GABAergic dendrites, some small (1–2 μm) and medium-sized (3–4 μm) unlabelled dendrites form many synapses with GABAergic terminals (Fig. 5A, C, D), although many unlabelled dendrites of all sizes are mainly contacted by terminals that form asymmetric synapses.

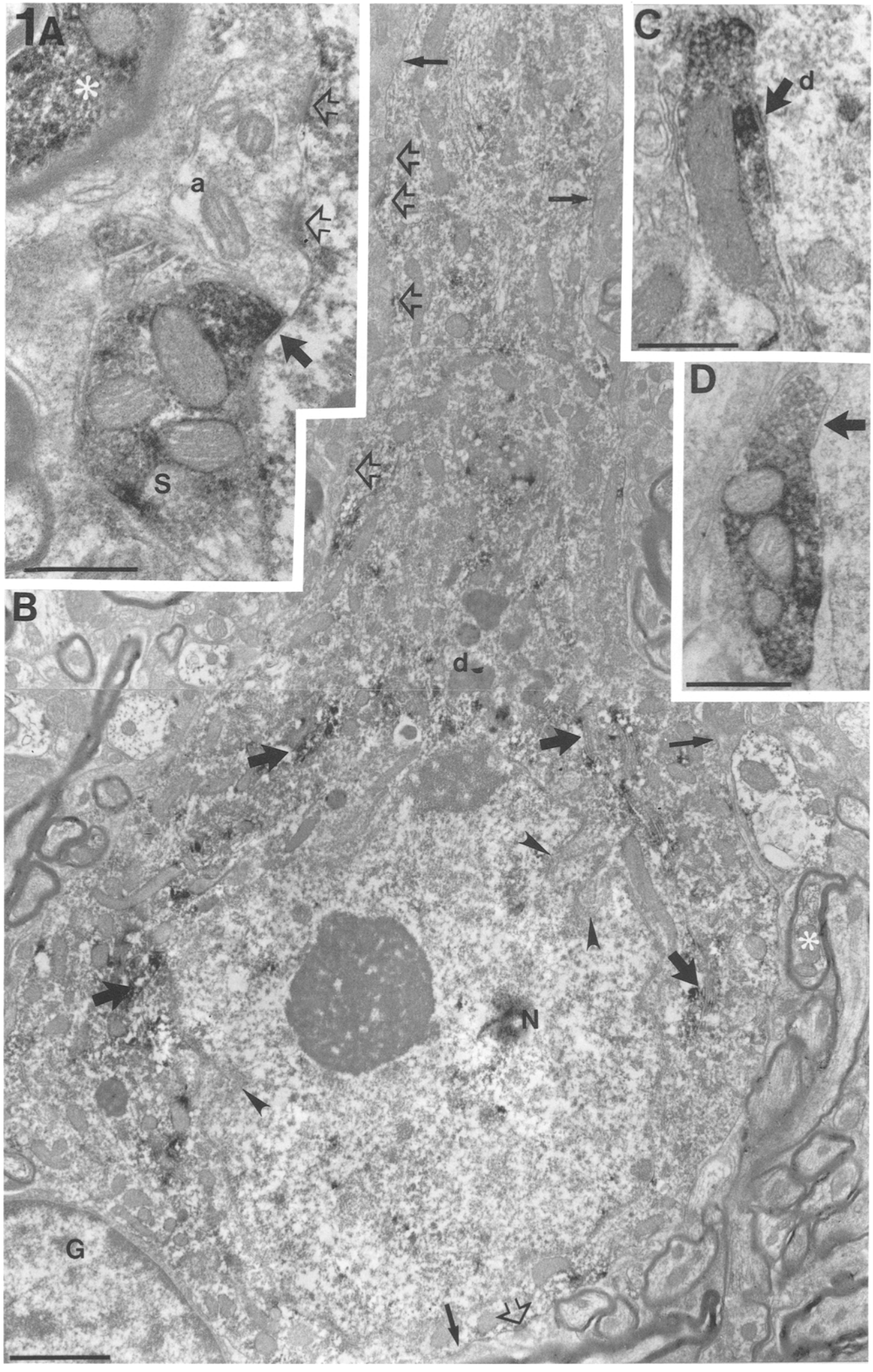
GABAergic axons

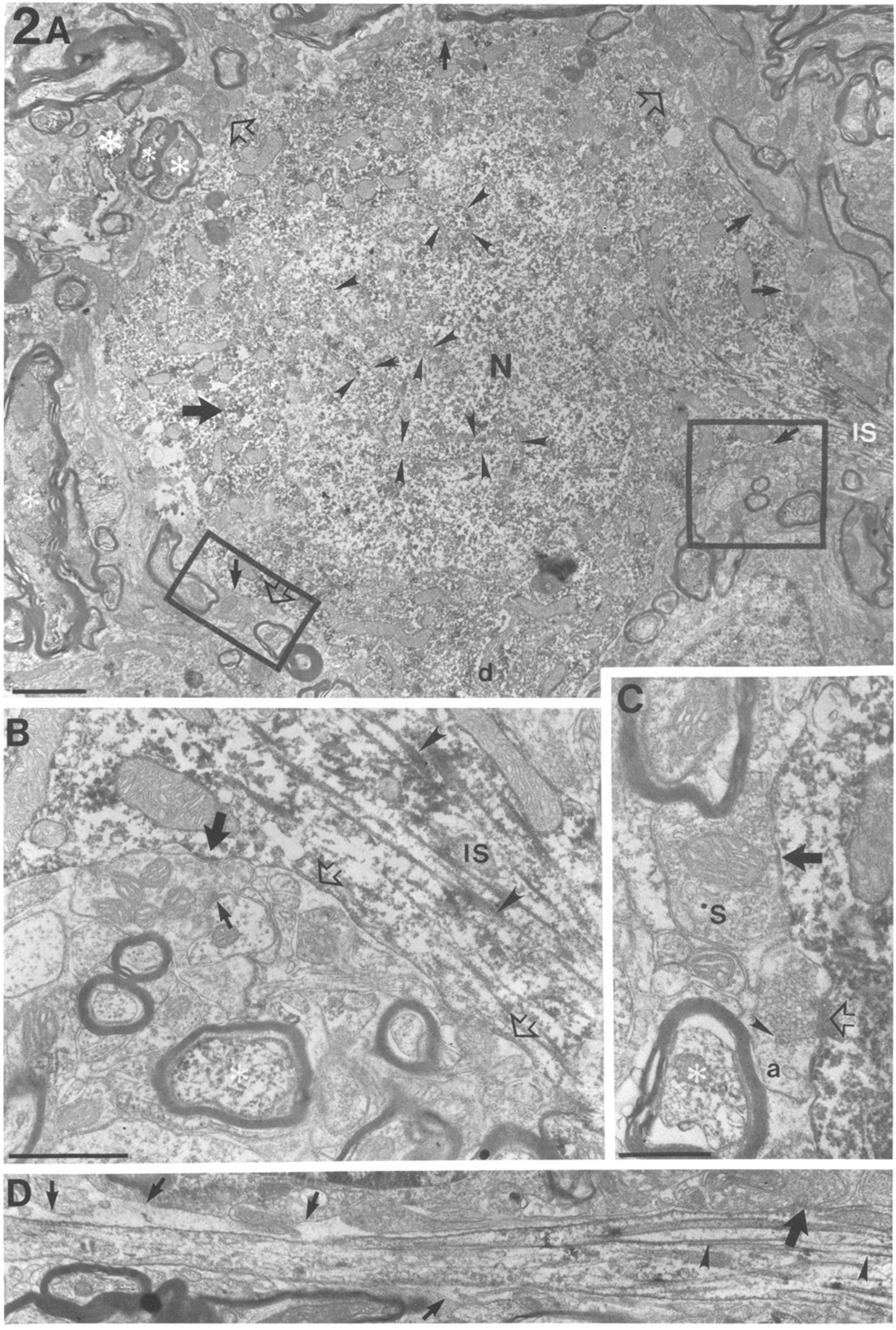
GABAergic terminals contain pleomorphic or flattened vesicles and make symmetric synapses with somata, proximal and distal dendrites of both immunoreactive and non-immunoreactive profiles. All immunoreactive terminals form symmetric synapses, although not all terminals forming symmetric synapses are immunoreactive. Terminals frequently contact more than one target in a given section (Figs 2B, 5C). GABAergic, myelinated axons (0.5–2.0 μm in diameter, including their myelin sheaths) are abundant (Figs 1A, 2B, 2C, 5B), and some terminate on the somata of small neurons (Fig. 5B).

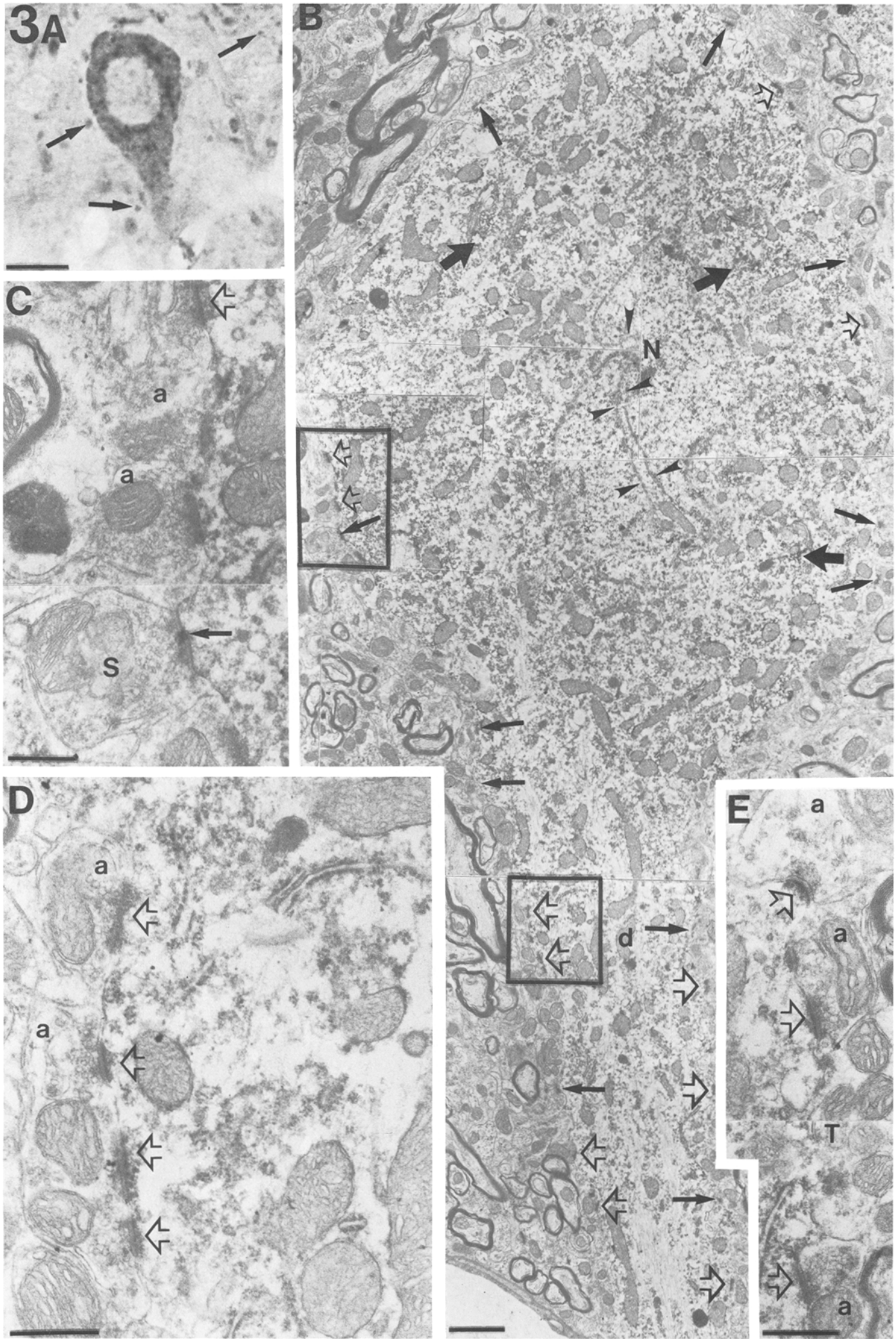
Fig. 1. (A) Examples of synapses on the soma and proximal dendrite of a GABAergic neuron similar to the one shown in Fig. 1B. Note that the immunoreactive terminal (S) contains several mitochondria and appears to form a symmetric synapse (arrow). The adjacent non-immunoreactive terminal (a) forms asymmetric synapses (open arrows). A GAD-positive myelinated axon (asterisk) is present in the adjacent neuropil. Scale bar: 0.5 μm. (B) A small, GAD-positive neuron, approximately 13 μm in diameter, with a round soma, infolded (arrowheads) nucleus (N), prominent nucleolus and stout proximal dendrite (d). The large arrows in the cytoplasm point to the immunoreaction product. Both symmetric (thin arrows) and asymmetric (open arrows) synapses occur on the soma and proximal dendrite. The soma is also apposed by glia (G) and myelinated axons, some of which are GAD-positive (asterisk). Scale bar: 2 μm. (C) An example of a GAD-positive axon terminal forming a symmetric synapse (arrow) with an immunoreactive dendrite (d). Note that the shape of the terminal is more elongated than the immunoreactive terminal shown in Fig. 1A. Scale bar: 0.5 μm. (D) An example of a GABA-positive terminal that appears to form a symmetric synapse (arrow) with a non-immunoreactive cell body. Scale bar: 0.5 μm.

Fig. 2. (A) A medium-sized, GABA-positive neuron, 18 μm in diameter, has a round soma, centrally located, infolded (arrowheads) nucleus (N) with an emerging dendrite (d) and axon initial segment (IS). This neuron receives eight axosomatic synapses from terminals that form asymmetric (open arrows) or symmetric (small arrows) synapses (not apparent at this magnification; see Fig. 2C for an enlargement of two of these contacts). The large arrow in the cytoplasm points to immunoreaction product. The region of the axon hillock enclosed in the box is shown at higher magnification in Fig. 2B. The region of the cell body enclosed in the box is shown at higher magnification in Fig. 2C. Myelinated axons (asterisk) are present in the adjacent neuropil. Scale bar: 2 μm. (B) Enlargement of the axon hillock and initial segment (IS) of the neuron in Fig. 2A, showing the typical fasciculation of microtubules (arrowheads) and dense undercoating. A terminal containing pleomorphic vesicles forms symmetric synapses with the axon hillock (large arrow) and an adjacent small dendrite (small arrow). The density associated with the axon hillock contact is greater because immunoreaction product is associated with the plasma membrane at that site. A glial sheath contacts the initial segment opposite the open arrows. A myelinated GABAergic axon (asterisk) is also present in the adjacent neuropil. Scale bar: 1 μm. (C) Enlargement of a portion of the cell body and axosomatic synapses in Fig. 2A. One terminal (a) contains round vesicles and forms an asymmetric synapse (open arrow); a dense core vesicle (arrowhead) is present in this terminal. An adjacent terminal (S) contains pleomorphic vesicles and appears to form a symmetric synapse (arrow). A myelinated, GABA-positive axon (asterisk) is present in the adjacent neuropil. Scale bar: 0.5 μm. (D) An axon initial segment with its fasciculation of microtubules (arrowheads) from the same type of neuron as shown in Fig. 2A. The portion of the initial segment proximal to the cell body receives a symmetric synapse (large arrow). A glial profile (small arrows) apposes the initial segment beginning approximately 3–5 μm from the cell body and continues distally more than 15 μm from the soma. Scale bar: 1 μm.

Fig. 3. (A) Light micrograph of a semithin section showing a GAD-positive neuron with a fusiform soma and stout dendrite. The perikaryal cytoplasm is packed with immunoreaction product, whereas the nucleus is unstained. GAD-positive puncta in the adjacent neuropil are indicated by arrows. Scale bar: 10 μm. (B) Electron micrograph of the same medium-sized neuron shown in Fig. 3A. This neuron displays a centrally located, highly infolded (arrowheads) nucleus (N). Terminals forming both asymmetric (open arrows) and symmetric (small arrows) synapses contact the soma (see enlargement in Fig. 3C for examples). Large arrows in the cytoplasm indicate reaction product. The boxed region of the soma indicates the location of the high magnification view shown in Fig. 3C. The dendrite (d) was followed approximately 30 μm from the soma, but only a portion is shown here. It is contacted by many terminals that form asymmetric synapses as well as a few that form symmetric synapses. The boxed region of this dendrite is enlarged in Fig. 3D. Scale bar: 2 μm. (C) Enlargement of a region of the cell body indicated by the top box in Fig. 3B. A terminal (S) containing pleomorphic vesicles forms a symmetric synapse with the soma (arrow). Two adjacent terminals (a) contain round vesicles and the top one forms an asymmetric synapse (open arrow). Scale bar: 0.5 μm. (D, E) Enlargements of axodendritic synapses from the region indicated by the lower box in Fig. 3B and from a region approximately 25 μm from the cell body, respectively. Both regions display numerous axon terminals (a) that form asymmetric axodendritic synapses (open arrows) and contain round vesicles. The terminals vary in size, and the number of active sites for each terminal ranges from one to three active zones indicating that they are probably perforated synapses. In Fig. 3E, one of the terminals (T) displays no active sites. Scale bars: 0.5 μm.


1A
a
S
C
d
D
B
d
N
G


2A
N
IS
d
B
IS
C
S
a
D


3A
B
C
D
E
a
a
S
N
d
a
a
a
a
T
a

Two different shapes of labelled terminals occur in the IC. One type is elongated in shape and approximately 0.5 to 1.5 μm in size, with the long axis of the terminal apposed to the postsynaptic surface. This type of terminal contacts both somata and dendrites (Fig. 1C, D) and was occasionally observed in continuity with its myelinated, preterminal axon (Fig. 5B). Round terminals are observed (1.0–2.5 μm in diameter) and are much more common than the elongated types (Figs 1A, 5A, C–E). They form synapses with both somata and dendrites.

Axon initial segments of GABAergic neurons were reconstructed from serial sections up to 15–20 μm from the cell body. The initial segments of the different subclasses of GABAergic neuron had similar features. They were approximately 1 μm in diameter at their origin, and the hillock region received only one or two symmetric synapses. At a distance of 3–5 μm from the cell body the initial segment became apposed by the profile of a glial sheath which extended as far as the segments could be traced (Fig. 2B, E). None of the initial segments became myelinated during the first 15–20 μm from the cell body, but it is possible that a myelin sheath may begin more distal to the soma than we were able to trace. The initial segments usually project straight out from the cell body, but occasionally an initial segment emerged from the soma and curled around the contours of the cell body. Other than the orientation with respect to the cell body (not shown), all other features were similar.

Discussion

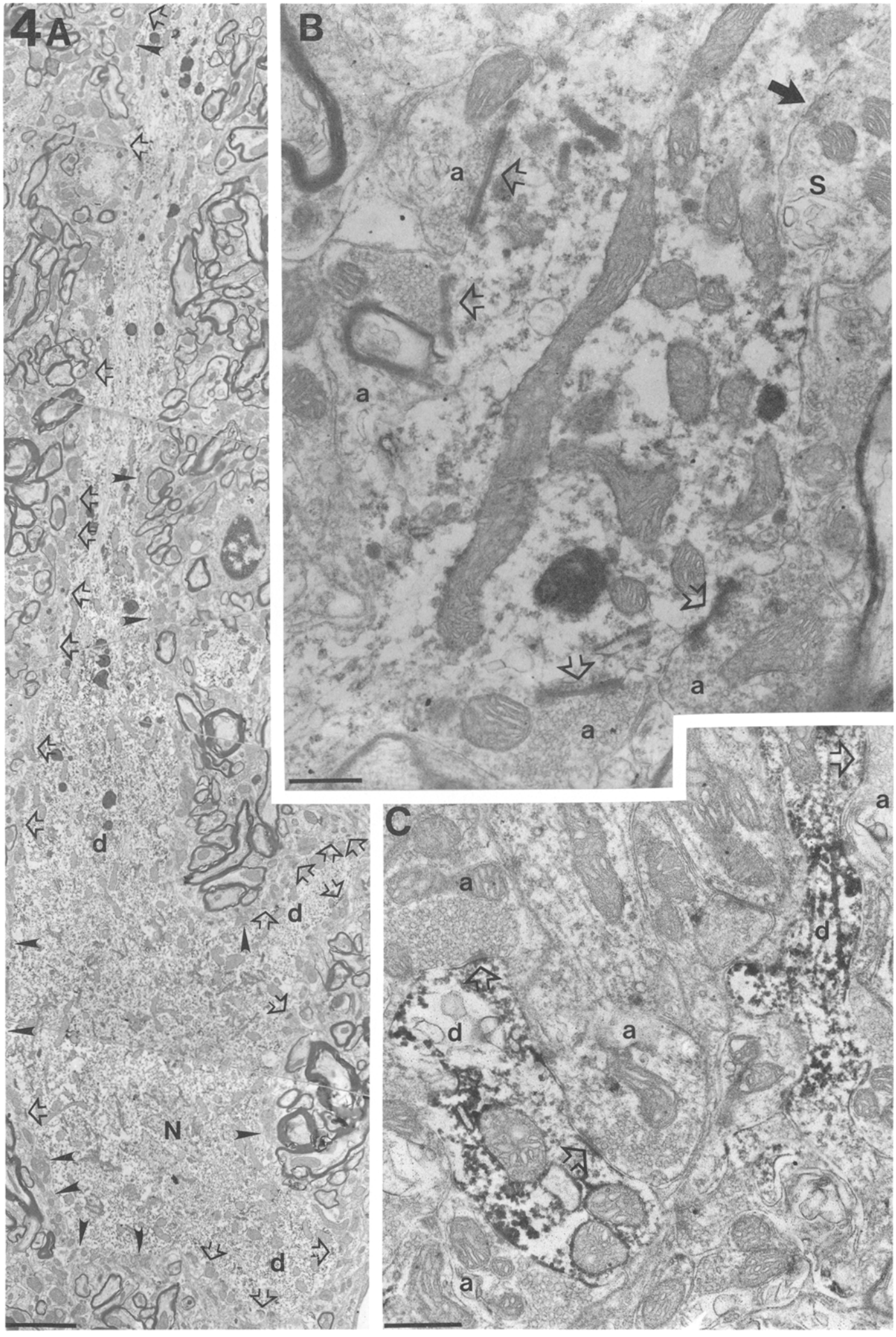
GABAergic neurons, dendrites and terminals are abundant in the ventral–lateral portion of the ICCN. These findings support the role of GABA as an important inhibitory neurotransmitter in this region.

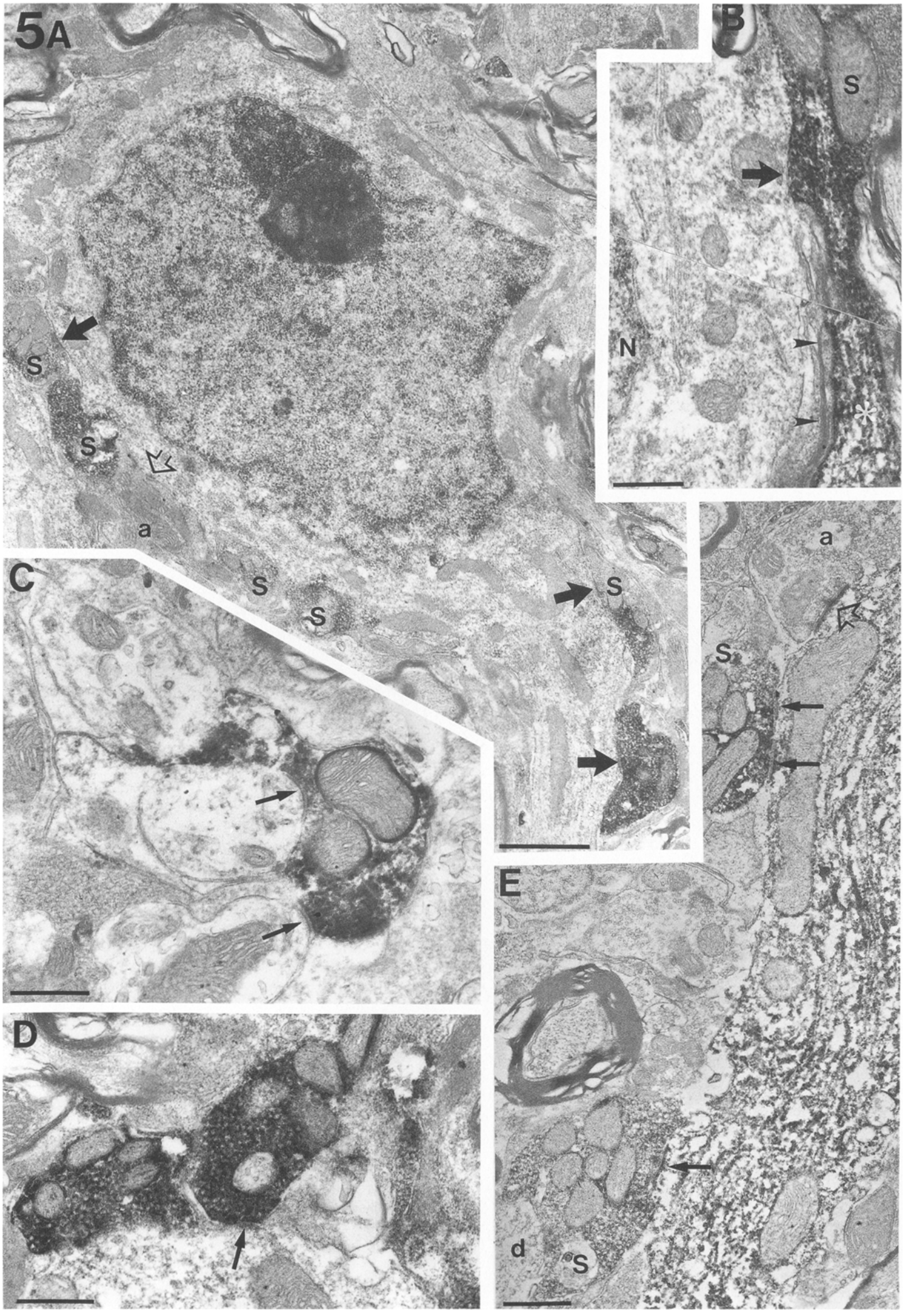
Neurons

The GABAergic multipolar neurons described in this study display the same ultrastructural features as those multipolar neurons reported in our earlier study of the rat ICCN (Ribak & Roberts, 1986). They also correspond to the multipolar cells previously described in ultrastructural studies of the cat by Rockel & Jones (1973a, b) and the stellate neurons described in light microscopic studies by Faye-Lund & Osen (1985) and Oliver & Morest (1985). Rockel & Jones (1973a, b) suggested that small and medium-sized multipolar neurons probably correspond to local circuit neurons because their axons are very thin and they were not able to trace the axons far from the cell bodies in Golgi material. Although there is no direct evidence for this suggestion, subsequent Golgi and retrograde tracing investigations have agreed that the small and medium-sized multipolar neurons are most probably local circuit neurons (Oliver, 1984; Faye-Lund & Osen, 1985; Oliver & Morest, 1985). Since these neurons form the majority of the population of GABAergic neurons within the ICCN, it is likely that most GABAergic neurons are local circuit neurons. Therefore, these GABAergic neurons are probably the source of many of the immunoreactive terminals in the ICCN.

Large multipolar neurons represent a small subclass of the GABAergic neuronal population in the ICCN. It appears that there are two types of large multipolar neuron, one subclass that is GABAergic and another that was unlabelled in our preparations and probably contains an excitatory neurotransmitter. This conclusion is based partially on the studies by Oliver (1984) and Morest (1975) which showed that some large multipolar neurons project to the medial geniculate body (MGB), and that projection neurons in the IC which terminate in the MGB form exclusively asymmetric synapses. If all GABAergic neurons in the ICCN have terminals that form symmetric synapses, as suggested by the present study, it is likely that the large multipolar neurons which are GABAergic do not project to the MGB. Thus, it is possible that there are two types of large multipolar neuron which have similar morphologies but contain different transmitters and have different targets. Unfortunately, there do not appear to be any ultrastructural characteristics that can be used

Fig. 4. (A) An example of a large, GAD-positive, multipolar neuron with three dendrites (d) extending from the soma. This section is not through the centre of the neuron and thus the diameter of the cell is only 20 μm through this particular section. However, this neuron displays the typical characteristics of a large neuron: highly infolded, eccentrically placed nucleus (N) and many terminals per unit area apposed to the soma with most forming symmetric synapses (arrowheads) (Ribak & Roberts, 1986). In contrast, the dendrites are contacted by terminals that form predominantly asymmetric synapses (open arrows) as determined by an analysis of enlarged electron micrographs. Note that there are many electron-dense, polymorphic lysosomes located in the proximal portion of the dendrite, but none in the soma. Scale bar: 5 μm. (B) A large GAD-positive dendrite receives a similar pattern of axodendritic synapses as the proximal dendrites from the neuron in Fig. 4A. Most terminals (a) contain round vesicles and form asymmetric synapses (open arrows). However, some terminals (S) which contain pleomorphic vesicles form symmetric synapses (solid arrow). Scale bar: 0.5 μm. (C) Some small GABA-positive dendrites (d) receive a similar pattern of synapses as the proximal dendrites shown in Fig. 4A and 4B in that most of the terminals (a) that contact them form asymmetric synapses (open arrows). Scale bar: 0.5 μm.


4A
B
a
S
a
a
a
d
d
N
d
C
a
a
d
d
a
a


5A
S
S
a
B
S
N
C
S
S
S
a
S
E
d
S
D

currently to distinguish these two types of neuron. The cell bodies of these large neurons in the rat all have similar ultrastructural features, e.g. many axosomatic synapses, a highly infolded, eccentrically located nucleus and many lysosomes (Ribak & Roberts, 1986). Although the target of the large multipolar GABAergic neurons is not known at this time, possible targets could be the contralateral IC or the dorsal cochlear nucleus (DCN). Since there is a high concentration of GABAergic terminals in the DCN (Moore & Moore, 1984; Mugnaini, 1985; Roberts *et al.*, 1985a) and lesions of the dorsal acoustic stria reduce the levels of GABA in DCN (Davies, 1981; Potashner *et al.*, 1985), a GABAergic projection to DCN is possible.

A small number of medium-sized neurons that have fusiform somata also are GABAergic. This finding suggests a similar situation as that for the large multipolar neurons. The morphological features of the fusiform somata are reminiscent of the disc-shaped (Faye-Lund & Osen, 1985; Oliver & Morest, 1985; Ribak & Roberts, 1986) or principal cell (Rockel & Jones, 1973a, b). However, as previously argued above, they probably do not project to the MGB. Oliver (1984) described medium-sized, disc-shaped projection cells as having smooth nuclear envelopes. Our data indicate that the GABAergic neurons of this type have highly infolded nuclear membranes, whereas non-immunoreactive, fusiform neurons have smooth nuclear membranes. Therefore, the nuclear features might provide a criterion to distinguish GABAergic from non-GABAergic, fusiform, medium-sized neurons in normal ultrastructural preparations.

Terminals

Since a large number of small and medium-sized multipolar neurons are GABAergic and are probably local circuit neurons, many GABAergic terminals in the ICCN probably have an intrinsic origin. However, it is unclear which type of GABAergic neuron gives rise to certain terminals, such as those found associated with large neuronal somata. A combined Golgi–electron microscopic analysis is necessary to demonstrate the intrinsic connections. The observation that GABAergic terminals do not all have the same shape may suggest that there is more than one source of these terminals.

Many studies indicate that some of the GABAergic terminals in the ICCN may arise from extrinsic sources. Since the present study was limited to the ventral–lateral portion of the ICCN, some terminals may arise from neurons located in lower brainstem auditory centres (Adams, 1979). For example, Adams & Mugnaini (1984) have reported that the dorsal nucleus of the lateral lemniscus (DNLL) contains exclusively GABAergic neurons and have deduced that GABAergic neurons in DNLL project to the IC. Although this study was based on preparations obtained from cats and the present study was made in the rat, the DNLL in rodents is very similar to that in the cat, in that it contains a high proportion of GABAergic neurons (Mugnaini & Oertel, 1985; Roberts *et al.*, 1985a, b; Thompson *et al.*, 1985). In contrast to these studies, Jones & Rockel (1972) have reported that lesions of the lateral lemniscus produced degeneration only in terminals which formed asymmetric synapses and contained round vesicles. However, the DNLL was probably spared in that study because its location is close to the IC and the investigators would not have wanted to damage the IC. Therefore, it is reasonable to assume that the data of Jones & Rockel (1972) do not contradict the hypothesis proposed by Adams & Mugnaini (1984) for the GABAergic projection from DNLL to the ICCN.

GABAergic terminals form synapses with GABAergic neurons and dendrites. The source of these terminals may be from recurrent collaterals or from other intrinsic or extrinsic GABAergic neurons. Thus, GABAergic neurons may receive synaptic contacts from other GABAergic local circuit neurons or from ascending GABAergic projections.

Although many terminals that have flattened or pleomorphic vesicles contained immunoreactivity, some did not. There are several possibilities for this

Fig. 5. (A) An example of a small, non-immunoreactive cell body. Note the large nucleus to cytoplasm ratio, irregular, although not infolded, nuclear membrane and prominent nucleolus. The soma and proximal dendrite are contacted by several immunoreactive terminals (S) and one of these forms a symmetric synapse (arrows). A non-immunoreactive terminal (a) forms an asymmetric axosomatic synapse (open arrows). Many GABA-positive terminals form symmetric synapses with the proximal dendrite as well as with the distal dendrite (not shown). Scale bar: 2 μm. (B) A GAD-positive, myelinated axon terminates on a non-immunoreactive neuron (N, nucleus) similar to the one shown in Fig. 5A. The preterminal axon (asterisk) displays paranodal myelin (arrowheads), and the terminal (S) appears to form a symmetric synapse (arrow) with the soma. Scale bar: 1 μm. (C) Another GABA-positive terminal forms symmetric synapses (arrows) with two adjacent, small, non-immunoreactive dendrites. Scale bar: 1 μm. (D) A portion of a medium-sized, non-immunoreactive dendrite is apposed by two GABA-positive terminals, and one of these forms a symmetric synapse (arrow). Scale bar: 0.5 μm. (E) A large, GABA-positive dendrite is contacted by both immunoreactive terminals (S) that form symmetric synapses (arrows) as well as an unlabelled terminal (a) which forms an asymmetric synapse (open arrow). The terminal in the lower left hand corner also makes a synapse on an adjacent dendrite (d). Scale bar: 0.5 μm.

latter observation. First, the use of colchicine, which blocks axonal transport, diminishes the number of labelled puncta. Therefore, it is possible that some of these terminals are indeed GABAergic, but they lack detectable levels of GAD or GABA in the terminals because these substances are trapped in the soma. Second, it is possible that some of these terminals may contain a transmitter other than GABA. Adams & Mugnaini (1985b) have localized cholecystokinin, substance P, met-enkephalin and neurotensin to cell bodies, fibres and terminals in the IC. Since some neuropeptides in other brain regions are found in terminals that form symmetric synapses (Connor & Peters, 1984; Hendry *et al.*, 1984), these substances may be found in the unlabelled terminals that form symmetric synapses in the IC. Alternatively, the lack of immunoreactivity in some of the terminals that form symmetric synapses may be a result of incomplete penetration of antibody. This is an unrelenting problem in immunocytochemistry because immunostaining is often compromised when attempts are made to obtain adequate fixation.

If unlabelled profiles consistently have different characteristics from labelled profiles, we can speculate about possible functions or patterns. For instance, all immunoreactive neurons in the ICCN have infolded nuclear envelopes, although not all neurons with infolded nuclei are labelled. In contrast, neurons with smooth nuclear envelopes never appeared to be labelled. Therefore, an infolded nucleus may be characteristic, although not diagnostic, for GABAergic neurons. In addition, some unlabelled profiles of dendrites are contacted by a larger number of immunoreactive terminals than GABAergic dendrites. If the unlabelled dendrites are indeed not GABAergic, then these data suggest that there is a greater amount of inhibition onto non-GABAergic dendrites than onto these GABAergic dendrites. If, however, the unlabelled profiles are GABAergic, then these data suggest that there are at least two patterns of synapses with GABAergic cells.

Acknowledgements

The authors wish to thank Yashu Jhurani and Margot Brundage for their expert technical assistance and Dr Wolfgang H. Oertel for supplying the GAD-antiserum. This study was supported by NIH Grant NS–15669, a Klingenstein Fellowship (CER) and a grant from the Epilepsy Foundation of America (RCR).

References

ADAMS, J. C. (1979) Ascending projections to the inferior colliculus. *Journal of Comparative Neurology* **183**, 519–38.

ADAMS, J. C. (1984) GAD-like immunoreactivity in the ventral cochlear nucleus. *Society for Neuroscience Abstracts* **10**, 393.

ADAMS, J. C. & MUGNAINI, E. (1984) Dorsal nucleus of the lateral lemniscus: a nucleus of GABAergic projection neurons. *Brain Research Bulletin* **13**, 585–90.

ADAMS, J. C. & MUGNAINI, E. (1985a) GAD-like immunoreactivity in the ventral nucleus of the lateral lemniscus. *Anatomical Record* **211**, 5A.

ADAMS, J. C. & MUGNAINI, E. (1985b) Patterns of immunostaining with antisera to peptides in the auditory brainstem of cat. *Society for Neuroscience Abstracts* **11**, 32.

CONNOR, J. R. & PETERS, A. (1984) Vasoactive intestinal polypeptide-immunoreactive neurons in rat visual cortex. *Neuroscience* **12**, 1027–44.

CONTRERAS, N. E. I. R. & BACHELARD, H. S. (1979) Some neurochemical studies on auditory regions of mouse brain. *Experimental Brain Research* **36**, 573–84.

DAVIES, W. E. (1981) The effect of lesions of the dorsal acoustic stria on the levels of glutamic acid decarboxylase and GABA transaminase in the inferior colliculus and cochlear nucleus of the guinea pig. *Neurochemistry International* **3**, 343–7.

FAINGOLD, C. L., GEHLBACH, G. & CASPARY, D. M. (1985) Effects of GABA on inferior colliculus neuronal responses to acoustic stimuli. *Society for Neuroscience Abstracts* **11**, 247.

FAYE-LUND, H. & OSEN, K. K. (1985) Anatomy of the inferior colliculus in rat. *Anatomy and Embryology* **171**, 1–20.

FISHER, S. K. & DAVIES, W. E. (1976) The presence and role of gamma-aminobutyric acid (GABA) in the guinea pig auditory system. *Journal of Neurochemistry* **27**, 1145–55.

GORENSTEIN, C., BUNDMAN, M. C., LEW, P. J., OLDS, J. L. & RIBAK, C. E. (1985) Dendritic transport. I. Colchicine stimulates the transport of lysosomal enzymes from cell bodies to dendrites. *Journal of Neuroscience* **5**, 2009–17.

GORENSTEIN, C. & RIBAK, C. E. (1985) Dendritic transport. II. Somatofugal movement of neuronal lysosomes induced by colchicine: evidence for a novel transport system in dendrites. *Journal of Neuroscience* **5**, 2018–27.

HENDRY, S. H. C., JONES, E. G. & EMSON, P. C. (1984) Morphology, distribution, and synaptic relations of somatostatin- and neuropeptide Y-immunoreactive neurons in rat and monkey neocortex. *Journal of Neuroscience* **4**, 2497–517.

JONES, E. G. & ROCKEL, A. J. (1972) Observations on complex vesicles, neurofilamentous hyperplasia and increased electron density during terminal degeneration in the inferior colliculus. *Journal of Comparative Neurology* **147**, 93–118.

MOORE, J. K. & MOORE, R. Y. (1984) GAD-like immunoreactivity in the cochlear nuclei and superior olivary complex. *Society for Neuroscience Abstracts* **10**, 843.

MOREST, D. K. (1975) Synaptic relationships of Golgi type II cells in the medial geniculate body of the cat. *Journal of Comparative Neurology* **162**, 157–94.

MUGNAINI, E. (1985) GABA neurons in the superficial layers of the rat dorsal cochlear nucleus; light and electron microscopic immunocytochemistry. *Journal of Comparative Neurology* **235**, 61–81.

MUGNAINI, E. & OERTEL, W. H. (1985) An atlas of the distribution of GABAergic neurons and terminals in the rat CNS as revealed by GAD immunohistochemistry. In *Handbook of Chemical Neuroanatomy*, Vol. 4, *GABA and Neuropeptides in the CNS*, Part I. (edited by BJÖRKLUND, A. & HÖKFELT, T.), pp. 547–53. New York: Elsevier.

OERTEL, W. H., SCHMECHEL, D. E., MUGNAINI, E., TAPPAZ, M. L. & KOPIN, I. J. (1981) Immunocytochemical localization of glutamate decarboxylase in rat cerebellum with a new antiserum. *Neuroscience* **6**, 2715–35.

OLIVER, D. L. (1984) Neuron types in the central nucleus of the inferior colliculus that project to the medial geniculate body. *Neuroscience* **11**, 409–24.

OLIVER, D. L. & MOREST, D. K. (1985) The central nucleus of the inferior colliculus of the cat. *Journal of Comparative Neurology* **222**, 237–64.

POTASHNER, S. J., LINDBERG, N. & MOREST, D. K. (1985) Uptake and release of γ-aminobutyric acid in the guinea pig cochlear nucleus after axotomy of cochlear and centrifugal fibers. *Journal of Neurochemistry* **45**, 1558–66.

RIBAK, C. E. & ROBERTS, R. C. (1986) The ultrastructure of the central nucleus of the inferior colliculus of the Sprague–Dawley rat. *Journal of Neurocytology* **15**, 421–38.

RIBAK, C. E., VAUGHN, J. E. & SAITO, K. (1978) Immunocytochemical localization of glutamic acid decarboxylase in neuronal somata following colchicine inhibition of axonal transport. *Brain Research* **140**, 315–32.

ROBERTS, R. C., RIBAK, C. E., KITZES, L. M. & OERTEL, W. H. (1985a) Regional distribution of GABAergic neurons and axon terminals in the brainstem auditory nuclei of the gerbil. *Anatomical Record* **211**, 161A.

ROBERTS, R. C., RIBAK, C. E. & OERTEL, W. H. (1985b) Increased numbers of GABAergic neurons occur in the inferior colliculus of an audiogenic model of genetic epilepsy. *Brain Research* **361**, 324–38.

ROBERTS, R. C., RIBAK, C. E., PETERSON, G. M. & OERTEL, W. H. (1984) Increased numbers of GABAergic neurons in the inferior colliculus of the genetically epilepsy prone rat. *Society for Neuroscience Abstracts* **10**, 410.

ROCKEL, A. J. & JONES, E. G. (1973a) The neuronal organization of the inferior colliculus of the adult cat. I. The central nucleus. *Journal of Comparative Neurology* **147**, 11–60.

ROCKEL, A. J. & JONES, E. G. (1973b) Observations on the fine structure of the central nucleus of the inferior colliculus of the cat. *Journal of Comparative Neurology* **147**, 61–92.

SOMOGYI, P., HODGSON, A. J., SMITH, A. D., NUNZI, M. G., GORIO, A. & WU, J.-Y. (1984) Different populations of GABAergic neurons in the visual cortex and hippocampus of cat contain somatostatin- or cholecystokinin-immunoreactive material. *Journal of Neuroscience* **4**, 2590–603.

TACHIBANA, M. & KURIYAMA, K. (1974) Gamma-aminobutyric acid in the lower auditory pathway of the guinea pig. *Brain Research* **69**, 370–74.

THOMPSON, G. C., CORTEX, A. M. & LAM, D. M.-K. (1985) Localization of GABA immunoreactivity in the auditory brainstem of guinea pigs. *Brain Research* **339**, 119–22.

VETTER, D. E. & MUGNAINI, E. (1984) Immunocytochemical localization of GABAergic elements in rat inferior colliculus. *Society for Neuroscience Abstracts* **10**, 1148.

WATANABE, T. & SIMADA, Z.-I. (1973) Pharmacological properties of cats collicular auditory neurons. *Japanese Journal of Physiology* **23**, 291–308.

ZAHM, D. S., ZABORSKY, L., ALONES, V. E. & HEIMER, L. (1985) Evidence for the coexistence of glutamate decarboxylase and met-enkephalin immunoreactivities in axon terminals of rat ventral pallidum. *Brain Research* **325**, 317–21.